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Chlorogenic acid ameliorates isoproterenol-induced myocardial injury in rats by stabilizing mitochondrial and lysosomal enzymes

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ABSTRACT

This study was deliberated to aspire the effects of chlorogenic acid (CGA) against myocardial infarction (MI) induced by Isoproterenol (ISO), in a rat model. In the pathology of MI, enzymes released due to the mitochondrial and lysosomal lipid peroxidation play an integral role. Induction of rats with ISO (85 mg/kg BW) for 2 consecutive days resulted in a significant decrease in the activities of heart mitochondrial enzymes isocitrate dehydrogenase (ICDH), α-ketoglutarate dehydrogenase (α-KGDH), succinate dehydrogenase (SDH) and malate dehydrogenase (MDH). The activities of lysosomal enzymes (β-glucosidase, β-glucuronidase, α-galactosidase, β-galactosidase, cathepsin-B and cathepsin-D) were increased significantly in the heart tissue. A prominent expression of LDH 1 and LDH 2 isoenzymes in the serum were observed and changes in the Electrocardiographic (ECG) patterns were also recorded in the ISO-induced rats. The prior administrations of CGA (40 mg/kg BW) for 19 days markedly ameliorated ISO induced alterations in ECG and significantly restored the activities of all the above enzymes in the heart of ISO-induced rats, which substantiates the stress stabilizing action of CGA. Oral administration of CGA (40 mg/kg BW) to normal rats did not show any significant changes. These biochemical functional alterations were supported by the histology of heart (Massion's trichrome and Picrosirius red staining for collagen formation). Thereupon, this study shows that 40 mg/kg BW of CGA gives protection against ISO-induced MI and demonstrates that CGA has a significant effect in the protection of heart.

1. Introduction

Cardiovascular diseases (CVDs) willbe the most important cause of mortality in India by the year 2030, 23.3 million people will die annually from CVDs and it will persist as the most common threats to human life [1]. It is worthwhile to mention that MI continues to be the major cause of CVDs. It occurs as a result of increased myocardial metabolic demand and decreased supply of oxygen and nutrients via the coronary circulation to the myocardium, leading to cell injury [2]. Overproduction of catecholamines due to adrenergic overstimulation is believed to be a major cause of stress-induced cardiac dysfunction [3]. ISO, a β-adrenergic agonist and synthetic catecholamine is employed at sub-maximal dose as a non-invasive method to induce myocardial lesions in rodents [4]. Out of several mechanisms proposed for understanding ISO-induced myocardial injury, production of highly cytotoxic free radicals through autooxidation is widely accepted. It induces severe stress and loss of myocardial integrity through hypoxia, calcium overload and increased free radical production [5].

MI can be evaluated by ECG [6,7]. Alteration of ECG segments reflects MI which is a definite criterion for diagnosis of either clinical or experimental MI.

Mitochondria are the momentous sub cellular organelles for cellular oxidative process and also the prime source of reactive oxygen species (ROS) in the cell. Mitochondria are the foremost source of energy, which nourish cellular metabolism and integrity. They are the locus of energy production and electron transport chain and accomplish a vital biochemical process called oxidative phosphorylation. The decrease in oxygen supply during MI impairs energy production by mitochondria [8].

Lysosomes are cytoplasmic organelles present in animal tissues, which contain hydrolytic enzymes capable of degrading the cellular constituents. They also play a sizable role in secretion and transport process. The lysosomal membrane is a potential site for free radical attack and its leakage can play an initiative role in apoptosis induced by oxidative stress [9]. Cathepsin-D is a

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lysosomal aspartic protease and β −Glucuronidase enzyme release is frequently used as an indidicator of lysosomal membrane integrity [10].

Phytomedicine is being used for the prevention of many cardiac ailments such as heart failure, coronary insufficiency and atherosclerosis from times immemorial. Many epidemiological studies suggest the protective effect of specific groups of fruits and vegetables against cardiovascular disorders [11]. Natural compounds rich in antioxidants are of paramount importance in dealing with various pathological conditions. Prevention of CVDs is associated with intake of vegetables and fresh fruits. In recent years, a number of health benefits associated with the consumption of CGA-abundant foods and drinks have been elucidated from epidemiology investigations. CGA is one of the most abundant polyphenol compounds present in green coffee beans and it is also present in fruits, vegetables and plants. CGA is used as an additive in various beverages, cosmetics, tea products and foods as well as in medical substances. The current commercial sources of CGA are from extracts of plants such as *Lonicera japonica Thunb* and *Eucommia ulmoides Oliver* etc [12]. *Lonicera japonica Thunb* has been shown to display a wide spectrum of biological and pharmacological activities such as antioxidant, antibacterial, antiviral [13], anti-inflammatory [14]. CGA has gained considerable attention due to their wide range of biological and pharmacological properties. The antioxidant activity of CGA has been reported in our previous study [15] and it is also exhibits antihyperlipidimic [16], antiapopototic [17] and antimicrobial [18] activities. In certain settings, CGAs have been shown to reduce the relative risks of type 2 diabetes [19,20] obesity [21] Alzheimer's disease [22,23] eclampsia and stroke [24]. Till date, no study has been carried out regarding the effect of CGA on membrane damage and ECG alterations induced by ISO. So, an attempt was made to evaluate the cardioprotective activity of CGA in ISO-induced *iso*-isoinduced MI by evaluating the ECG and biochemical changes discussed below.

2. Materials and methods

2.1. Chemicals

Isoproterenol hydrochloride, CGA and all other chemicals were of analytical grade and chemicals required for sensitive biochemical assays were obtained from Sigma Chemicals, St. Louis, MO, USA.

2.2. Animals

Healthy adult male albino Wistar rats with a body weight ranging from 150 to 180 g, was procured from Central Animal House, Department of Experimental Medicine, Rajah Muthiah Medical College and Hospital, Annamalai University, and it was maintained in an air-conditioned room. Water and standard laboratory diet *ad libitum* was provided. The experiments were carried out as per the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), New Delhi, India. (Reg No. 160/1999/CPCSEA, Pro. No. 1100).

2.3. Preparation of CGA

CGA (40 mg/kg BW) was dissolved in distilled water and administered post orally (p.o) by intubation once in a day for 19 days. The effective dose of CGA (40 mg/kg BW) was selected based on dose dependent study of CGA carried out in our laboratory [15].

2.3.1. Induction of MI

ISO, 85 mg/kg BW was dissolved in physiological saline (0.9% NaCl) and injected subcutaneously (s.c) in the right thigh of the rat for two consecutive days, at an interval of 24 h [25,26] to induce MI.

2.4. Experimental design

A total number of 24 rats were randomly divided into four groups comprising of six animals in each group.

Group I	:	Normal
Group II	:	CGA (40 mg/kg BW, p.o for first 19 days)
GroupIII	:	ISO (85 mg/kg BW, s.c. on 20th and 21st day)
GroupVI	:	CGA (40 mg/kg BW, p.o for first 19 days) + ISO (85 mg/kg BW, s.c. on 20th and 21st day)

The total experimental duration was 21 days. At the end of the experimental period, ECG was recorded by using a 16-channel polygraph (Biopac Systems Inc., USA). Electrocardiographic parameters such as ST segment, R-amplitude, P wave, QRS complex, QT interval, R-R interval and Heart rate were recorded. On 22nd day the rats were sacrificed between 8:00 am and 9:00 am by cervical dislocation. Serum was separated and used for LDH isoenzymes assay and heart tissues were excised immediately, rinsed in ice-chilled saline and blotted with filter paper and weighed. All enzyme assays were done immediately.

2.5. Isolation of mitochondria from the heart tissue and the assessment of mitochondrial enzymes activities

Mitochondria rich fraction was isolated from heart tissue of different experimental animals by differential centrifugation as described in earlier reported literature [27]. Briefly, A 20% (w/v) homogenate was prepared in 0.25 M sucrose containing 0.05 M tris-HCl buffer and 5.0 mM EDTA. To remove cell debris, tissue fragments and cell nuclei (nuclei pellet), the homogenate was centrifuged at 600 x *g* for 10 min. The supernatant fraction was centrifuged (Himac SCP 70G, Hitachi, Japan) in a refrigerated centrifuge at 10,000 x *g* for 5 min at 4 °C to bring down the mitochondrial pellet. After centrifugation, the supernatant was poured off while the loose upper part of the mitochondrial pellet may come off as well. Most of the pellet, containing healthy mitochondria, was dense enough to remain behind. The white foamy material, near the top of the tube consists of lipids, were removed by wiping the inside of the tube with cotton. Because any mixing of lipids with the mitochondria suspension will cause them to uncouple (lose their ability to maintain a chemiosmotic gradient). After using a Pasteur pipette to remove the last bit of liquid, the remaining mitochondrial pellet was resuspended in specific volume of potassium chloride (KCl) and used for the estimation of various parameters. The activities of heart mitochondrial enzymes such as ICDH [28], SDH [29], MDH [30], α-KGDH [31] were analyzed.

2.6. Isolation of lysosomal fraction from the heart tissue and the assessment of activities of lysosomal enzymes

The lysosomal fraction of the heart tissue was isolated by the method of Jamieson and Robinson (1977) [32]. Fresh heart tissue was homogenized in ice-cold 0.25 M sucrose solution. The homogenate was filtered and centrifuged at 3000 × *g* for 10 min. The pellet was removed and rehomogenized and resuspended as before. The supernatants were combined and centrifuged again at 15,000 × *g* for 20 min. The lysosomal pellet was suspended in 1.15% KCl and homogenized and used for the assay of enzymes. The activities of lysosomal enzymes such as β − glucuronidase [33], β-glucosidase, α and β-galactosidase [34], Cathepsin-B [35] and Cathepsin-D [36] were analyzed.